PCT / SE 2004 / 000451

PRV

PATENT- OCH REGISTRERINGSVERKET
Patentavdelningen

Intyg
Certificate

Härmed intygas att bifogade kopior överensstämmer med de handlingar som ursprungligen ingivits till Patent- och registreringsverket i nedannämnda ansökan.

This is to certify that the annexed is a true copy of the documents as originally filed with the Patent- and Registration Office in connection with the following patent application.

(71) *Sökande* / *Applicant (s)* — *Isconova AB, Uppsala SE*

(21) *Patentansökningsnummer* / *Patent application number* — *0300795-2*

(86) *Ingivningsdatum* / *Date of filing* — *2003-03-24*

REC'D 14 APR 2004
WIPO PCT

Stockholm, 2004-03-26

För Patent- och registreringsverket
For the Patent- and Registration Office

Marita Öun

Avgift
Fee

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

PATENT- OCH REGISTRERINGSVERKET
SWEDEN

Postadress/Adress
Box 5055
S-102 42 STOCKHOLM

Telefon/Phone
+46 8 782 25 00
Vx 08-782 25 00

Telex
17978
PATOREG S

Telefax
+46 8 666 02 86
08-666 02 86

Composition comprising iscom particles and live micro-organisms

The present invention relates to the use of iscom particles as adjuvant for preparing of an antigenic composition, which comprises live micro-organisms and a composition comprising at least one iscom particle and one or more living micro-organisms.

Technical background

Today adjuvant are used to enhance the immunogenicity of antigens which are not replicating i.e. in so called killed or inactivated vaccines. Although, many vaccines contain several kinds of vaccine antigens in order to cover immune protection against several infectious diseases live and killed vaccine antigens are not mixed. One reason for that is that killed vaccines need adjuvant to enhance the effect of killed vaccines.

Live vaccines contain micro-organisms that replicate in the host. The vaccines micro-organism is closely related to the pathogen i.e. the micro-organisms that cause disease. Thus, the host is producing most of the vaccine antigens when replicating vaccine antigens are used resulting in high doses in the host.

Moreover, the production of vaccine antigens in the host over a period of time contributes also to make the live vaccines effective and even one dose often suffice to evoke long lasting immune protection. For certain pathogens there is no live micro-organism to represent them in a vaccine, either because the attenuation is not achieved, or that after the attenuation the micro-organism does not induce a potent immune protection.

It is of practical reasons and economical reasons to give all vaccines in one dose when there is a defined period for vaccination. Such a period is in the early childhood respectively newborn animals when up to seven different vaccines are given in one injection. Another such a period is before travel to exotic countries to induce immune

protection against exotic infections, i.e. against infectious diseases, that are not present in the home country.

Most vaccines in dogs are used in puppies and the first vaccination is carried just before or at the time for delivery to the new owner. There is a strong tendency, not to say desire, to avoid more than one primary vaccination and limit the number of re-vaccinations. Most vaccines in dogs are live partly due to the fact of the difficulty to combine live and killed vaccines. A dog vaccine today is mostly a multicomponent vaccine and composed to protect against up to seven different infectious diseases.

Examples of killed vaccines in dogs are rabies virus vaccines and a vaccine against Bordetella brochoseptica, and these vaccines are single vaccines. Rabies virus vaccines require adjuvant, and so far aluminium hydroxide is used which adsorb micro-organism and prevent thereby their replication.

Thus, there is a desire to be able to use killed and live vaccines mixed in a common formulation and that they should be compatible with each other in the formulation. Further, there is a need that adjuvant also could be present in the formulation without causing adverse effects.

It has now surprisingly turned out that iscoms and iscom matrix particles can be used as adjuvants for living organisms without affecting them negatively. This is contrary to (most) other commonly used adjuvants that decrease the capacity of the live micro-organisms to replicate.

Summary of the invention

The present invention relates to the use of iscom particles as adjuvant for preparing of an antigenic composition, which comprises live micro-organisms and a composition comprising at least one iscom particle and one or more living micro-organisms.

Several saponins formulated in iscom and iscom matrix have been tested for their effect on various viruses, which are involved in vaccine formulations. Live vaccine antigens were mixed with the adjuvant formulation and the mixture incubated for two or more hours. Thereafter, the capacity of the micro-organism to replicate in cell cultures or in a host, in this case in a chicken embryo, was measured. The iscom particles did not hamper the replication of the live micro-organisms and even enhanced proliferation in contrary to several other commonly used adjuvant, that were tested.

Detailed description of the invention

The present invention relates to the use of an iscom particle as adjuvant for preparing of an antigenic composition, which comprises at least one live micro-organism.

By live micro-organism we understand a micro-organism that can replicate in the host. The micro-organism must not be in a condition to affect the host severely. Therefore, preferably attenuated micro-organisms are used. Attenuation is known in the art and may be performed as described in New Vaccine Technologies (2001) Ed. Ronald W Ellis, Landes Bioscience, Georgetown, Texas, USA.

The live micro-organism may be any micro-organism of interest for use as an antigen for triggering or modulating the immune system. The micro-organisms may be chosen from viruses including smallpox virus, Japanese encephalitis virus, yellow fever vaccines, poliovirus vaccines, measles vaccines, rubella vaccines, mumps vaccines and trivalent vaccines including measles, mumps-rubella vaccines or even one more live virus vaccine i.e.varicella vaccine; gram + and gram- live bacterial vaccines including live attenuated mycobacterium bovis (BCG Tuberculosis Vaccine), live attenuated Salmonella typhi, live attenuated Shigella spp, live virulence-attenuated vibrio cholerae, pediatric.

Examples of live vaccines in animals, but not limited to the examples, are vaccines against canine distemper virus, canine parvovirus, canine adenovirus, parainfluenza 3 viruses in dogs and cattle, feline parvovirus.

The invention, especially relates to the use of the iscom particles together with live micro-organisms in a vaccine composition for eliciting an immune protection in a host treated with the vaccine.

Different species of micro-organisms may be used in the same composition comprising the iscom particles or in different compositions for co administration at the same event.

The iscom particles may also be used in a composition that further comprises at least one killed or inactivated micro-organism together with one or more live micro-organisms. Inactivation is known in the art and may be performed as described in New Vaccine Technologies (2001) Ed. Ronald W Ellis, Landes Bioscience, Georgetown, Texas, USA or as described by Rueda. P. et al. 2001. Vaccine 19 (2001) p. 726-734. Effect of different baculo virus inactivation procedures on the integrity and immunogenecity of Porcine Parvo virus - like particles.

Inactivated bacterial vaccines that include conjugate or sub-unit vaccines such as group Streptococci, group A Streptococci, Haemophilus influenzae, Neisseria meningitides, bordetella pertussis, Streptococcus pneumonia, Mycoplasma pneumonia. Examples of adult attenuated vaccine are those against cholera enterotoxic E coli, shigellosis etc.

Killed vaccines, but not limited to the examples are, for use in animals (dogs) rabiesvirus vaccines and a vaccines against Bordetella brochoseptica, vaccines against leptospirosis and respiratory syncytial and bovine virus diarrhoea virus, bovine herpes virus 1 in cattle, or influenza viruses in horse.

Examples of killed vaccines for use in humans are inactivated virus vaccines include tick-borne encephalitis-, rabies-, hepatitis A-, polio-, influenza viruses.

The invention also relates to the use of iscom particles whereby the antigenic composition further comprises one or more antigenic molecules.

The iscom particle may be an iscom or an iscom matrix particle or any subfragment thereof.

Iscom contains at least one glycoside, at least one lipid, and at least one kind of antigen substance or epitope. These substances may be of different kind such as proteins and peptides, glycoproteins and glycopeptides, carbohydrates etc.. These complexes enhance the immunogenicity of the included antigens and may also contain one or more immunomodulatory (adjuvant-active) substances. Iscoms may be prepared as described in EP 0 109 924 B1, EP 0 242 380 B1 and EP 0 180 546 B1.

Matrix contains at least one glycoside, which is an adjuvant-active substance and at least one lipid. Matrix has an immunoenhancing effect on co-administered antigenic substances, see EP 0 436 620 B1. Matrix may contain other immunostimulating and enhancing components than saponins e.g. lipopolysacharides (LPS), Lipid A,

Iscom particles containing such antigenic molecules integrated into the particle, coupled on to the particle or simply mixed into the composition may be used together with the live and/or inactivated micro-organisms.

The lipids used are particularly those described in the applicant's patent EP 0 109 942 B1 in particular on p. 3 and in patent EP 0 436 620 B1 on p. 7 lines 7-24. Especially sterols such as cholesterol and phospholipids such as phosphatidylethanolamin and phosphatidylcolin are used. Lipid-containing receptors that bind to the cell-binding components, such as glycolipids including the cholera toxin's receptor, which is the ganglioside GM1, and fucosed blood group antigen may be used. The cell-binding

components can then function as mucus targeting molecule and be bound to the lipid-containing substances through simply mixing them with complexes that contain them. Iscom complexes comprising such receptors and receptors are described in WO 97/30728

The glycoside in the iscom particles may be any glucoside. Preferred glucosides are described in EP 0 109 924 B1. Especially preferred are raw extract from *Quillaja Saponaria* Molina" (Dalsgaard, K. (1974), Arch. Gesamte Virusforsch, 44, 243.), or any subfraction thereof as described in PCT/US/88101842 to Kensil et al., Kensil, C.A. et al. (1991), J. Immunol., 146, 431, Kersten, G.F.A. et al. (1990). "Aspects of Iscoms. Analytical, Pharmaceutical and Adjuvant Properties; Thesis, University of Utrecht, EP 0 362 279 B2 and EP 0 555 276 B1

The saponin fractions according to the invention may be the A, B and C fractions described in WO 96/11711, the B3, B4 and B4b fractions described in EP 0 436 620 The fractions QA1-22 described in EP 0 3632 279 B2, Q-VAC (Nor-Feed, AS Denmark), *Quillaja Saponaria* Molina Spikoside (Isconova AB, Ultunaallén 2B, 756 51 Uppsala, Sweden)

The fractions QA-1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18-19-20-21 and 22 of EP 0 3632 279 B2, Especially QA-7, 17-18 and 21 may be used. They are obtained as described in EP 0 3632 279 B2, especially at page 6 and in Example 1 on page 8 and 9.

Preferably sub fractions A and C are used. It has surprisingly turned out that A-matrix and C-matrix enhances virus growth (see Example 2).

The term " one saponin fraction from *Quillaja Saponaria* Molina." is used throughout this specification and in the claims as a generic description of a semi-purified or defined saponin fraction of *Quillaja Saponaria* or a substantially pure fraction. It is important that the fraction does not contain as much of any other fraction to negatively

affect the good results that are obtained when the mixtures of iscom or iscom matrix comprising essentially one fraction is used. The saponin preparation may, if desired, include minor amounts for example up to 40% by weight, such as up to 30 % by weight, up to 25 % by weight, up to 20 % by weight, up to 15 % by weight, up to 10 % by weight, up to 7 % by weight, up to 5 % by weight, up to 2 % by weight, up to 1 % by weight, up to 0,5 % by weight up to 0,1 % by weight of other compounds such as other saponins or other adjuvant materials.

The antigenic molecules may be coupled on to the iscom matrix particle or simply mixed into the composition and used together with the live and/or inactivated micro-organisms.

The antigenic molecules which may be incorporated into or associated with the iscom matrix in accordance with this invention may be any chemical entity which can induce an immune response in an individual such as (but not limited to) a human or other animal, including but not limited to a humoral and/or cell-mediated immune response to bacteria, viruses, mycoplasma or other micro-organisms. The specific immunogen can be a protein or peptide, a carbohydrate, polysaccharide, a lipopolysaccharide or a lipopeptide; or it can be a combination of any of these.

Particularly, the specific antigenic molecule can include a native protein or protein fragment, or a synthetic protein or protein fragment or peptide; it can include glycoprotein, glycopeptide, lipoprotein, lipopeptide, nucleoprotein, nucleopeptide; it can include a peptide-peptide conjugate; it can include a recombinant nucleic acid expression product.

Examples of such immunogens are cited in EP 0 109 942 B1 and include, but are not limited to, those that are capable of eliciting an immune response against viral or bacterial hepatitis, influenza, diphtheria, tetanus, pertussis, measles, mumps, rubella, polio, pneumococcus, herpes, respiratory syncytial virus, haemophilias influenza, chlamydia, varicella-zoster virus, rabies or human immunodeficiency virus.

Any type of iscom particle, iscom matrix particle, live and inactivated microorgnism and antigenic substance may be used together in a composition for use as an antigenic or immune modulating agent according to the invention.

Also, one or more iscom particles, iscom matrix particles, live and inactivated micro-orgnisms and antigenic substances may be used together in a composition for use as an antigenic or immune modulating agent according to the invention

The invention also concerns a composition comprising at least one iscom particle and one or more living micro-organisms. The composition may be a vaccine, wherein the living micro-organism is a virus. Such a composition may further comprise one or more killed or inactivated micro-organisms. It may also comprise one or more antigenic molecules.

The composition may be used for animals within the veterinary medicine and for humans.

Pharmaceutical and veterinary medicine compositions according to the present invention, and for use in accordance with the present invention, may include, in addition to active ingredient, a pharmaceutically acceptable excipient, diluent, carrier, buffer, stabiliser, additive or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration.

A live vaccine antigen is generally freeze dried and before administration the vaccine antigens (live micro-organisms) are dissolved in a pharmaceutically acceptable solvent. The solubilized, or suspended vaccine antigens shall immediately be administered to the individual. Before administration the fries dried an antigen is dissolved/suspended in a solvent that may contain the adjuvant i.e. iscom matrix or

iscom formulation with or without the killed vaccine and/or antigenic substance(s). Alternatively before suspending/solubilizing of the fries dried component in the vaccine the solvent is mixed with the iscom matrix and/or iscom particles and/or the killed micro-organisms and/or the antigenic molecules.

The pharmaceutically acceptable solvent may be a buffer e.g. PBS.

The live micro-organisms are preferably supplied freeze dried separate from the adjuvant particles.

Thus, the invention also relates to a kit of parts comprising at least one compartment containing at least one living organism and at least one compartment containing at least one iscom particle.

A kit of parts may comprise different compartments e.g. one or more compartments comprising at least one freeze dried live micro-organism and at least one compartment comprising at least one iscom particle. The iscom particle is preferably dissolved or suspended in a pharmaceutical acceptable solvent.

Another embodiment according to the invention relates to a kit of parts, which further comprises also at least one inactivated micro-organism, which may be present in a further compartment or in the same compartment as one compartment containing at least one iscom particle.

When antigenic molecules are present they may be integrated into or coupled on to an iscom particle or mixed with one or more iscom matrix particles and kept in the same compartment.

The amount of antigenic substance, inactivated micro-organism and live micro-organism is dependent on the substance and micro-organisms used and the individual to be treated. The content of live micro-organism further depends on the constitution

of the micro-organism. For inactivated non living micro-organism the in small animals the low dose is 0.1 μg up to 100 μg, for large animals the low dose range from 10 μg up to 300 μg that said not to be limiting borders. In humans the dose ranges are 1 μg up to 200 μg not being the limiting border.

The invention will now be further described by way of non-limiting examples. All references mentioned herein are incorporated by reference.

Example 1

The following adjuvant formulations were tested for their effect on live virus to explore if they interfere with live virus antigen replication in the chicken embryo: A-matrix, C-matrix, 703 matrix crude spikoside matrix, oil adjuvant, aluminiumhydroxide, influenza virus iscoms and respiratory syncytial virus iscoms.

From a stock solution of influenza virus containing 10 log9 a dilution in PBS 10log6 was prepared as working dilution. To one ml of this virus dilution 50, 100 and 200 μg of each of the formulations was added. The virus – adjuvant mixtures were incubated for at least 2 hrs before 100 μl were injected into the allantois fluid of 11 days embryonated hens eggs. The allantoic fluid was harvested at day 18 of hatch. The level of virus replication was measured as embryo infectious dose 50 (EID50) i.e. the end point where 50% of the embryos are infected. The detection of infection, i.e. the presence virus in the allantois fluid from the embryonated egg, is based on the phenomenon that the influenza virus aggregates chicken red blood cells so called hemagglutination (HA).

Results

Four control groups, which included 7 to 10 embryos each, were infected with influenza virus that was not pre-incubated with any of the adjuvant formulations. The EID50 titres ranged between 10 log 9.2 to 10 9.5.

None of the matrix or iscom formulations mentioned above reduced the EID50 titres compared to the EID50 titres measured in the control groups. In contrast oil and aluminumhydroxide reduced the EID50 titres more than a 10log, which is unacceptable for blending with live vaccine antigens i.e. they can not be used in a vaccine containing live vaccine antigens. Thus, it is concluded that the matrix formulations are "compatible" for use in vaccines, which contain live micro-organisms.

Example 2

The following adjuvant formulations were tested to explore if they interfere with the replication of canine distemper virus (CDV): A-matrix, C-matrix, 703 matrix, spikoside matrix, Q-VAC matrix, oil adjuvant, aluminiumhydroxide, free saponin A, free saponin C, free 703 and free spikoside, influenza virus iscoms and respiratory syncytial virus iscoms.

From a stock solution of CDV containing 10 log5 a 10-fold dilution in virus medium without serum was prepared as working dilution. To one ml of this virus dilution 50, 100 and 200 µg of either of the adjuvant formulations was added. The virus–adjuvant mixtures were incubated for at least 2 hrs before 200 µl of the virus-adjuvant mixture in dilutions 10log-1 (calculated from stock virus) to dilution 10log –5 were allowed to adsorb for 1 to 2 hours at 37°C to vero cell cultures adherent to the 25 cm2 plastic surface in Costar flasks (No. 3055, Corning Inc., Corning, N.Y. 14831, USA).

Thereafter, virus suspensions respectively virus-adjuvant suspensions were removed as far as possible and cell culture medium containing 2% calf serum was added to each flask.

The level of virus replication was measured as tissue culture infectious dose 50 (TCID50), i.e. the end point where 50% of the tissue cultures are infected. The detection of infection, i.e. the presence virus in the tissue cultures is based on the cell destruction the virus is causing i.e. cytopatic effect (CPE). The specificity of the reaction was confirmed by immunoflurorescens or by neutralisation of recovered virus from the cell cultures. The cultures were followed and examined for 8? days when the virus controls showed 50 to 100% CPE (cell destruction), while uninfected cultures still had confluent layers of cells.

The virus controls included virus in the same dilutions treated in the same way as the virus–adjuvant mixtures, except that the virus suspensions were not mixed with the adjuvant formulations.

The cell controls were uninfected cells.

Each mixture and control assay was carried out in four replicates.

Results

The four virus controls, i.e. CDV that was not pre-incubated with any of the adjuvant formulations, titred out to 4.7 TCID50 10LOG titres.

A-matrix, C-matrix treated virus titred both out to 5.7 i.e. a ten fold higher titre than the virus control i.e. an un-expected increase of virus growth.

703 matrix (4.7), A + C matrix (4.7), Q-VAC matrix (4.5), free saponin A, influenza virus Iscoms (4.9) and respiratory syncytial virus Iscoms (4.4). The titres in brackets

show that all these formulations did not significantly differ from the titres of the virus control.

Spikoside matrix, free saponin C, free 703 and free spikoside, oil adjuvant and aluminiumhydroxide decreased more than a ten fold the virus titres compared to the virus control.

In concluson, Spikoside matrix, free saponin C, free 703 and free spikoside, oil adjuvant and aluminiumhydroxide can not be used together with live vaccine antigens, because they decrease the capacity to replicate, when mixed with the live vaccine antigens.

703 matrix, A + C matrix, Q-VAC matrix, free saponin A, influenza virus Iscoms and respiratory syncytial virus Iscoms can all be used as adjuvant in a vaccine containing live antigens.

A-matrix, C-matrix treated enhanced virus growth in cell cultures an un-expected result, which can lead to increased efficacy.

Patent claims

1. Use of an iscom particle as adjuvant for preparing of an antigenic composition, which comprises at least one live micro-organism.

2. Use according to claim 1, wherein the antigenic composition is a vaccine comprising at least one live virus.

3. Use according to claim 1and/or 2, wherein the antigenic composition further comprises at least one killed or inactivated micro-organism.

4. Use according to any of claim 1-3, wherein the antigenic composition further comprises one or more antigenic molecules.

5. Use according to any of claims 1-4, wherein the iscom particle is an iscom comprising at least one glycoside, at least one lipid and at least one hydrophobic protein or peptide-containing antigen.

6. Use according to any of claims 1-4, wherein the iscom particle is an iscom-matrix, comprising at least one glycoside and at least one lipid.

7. Use according to any of claims 1-6, wherein the iscom particle comprises at least one glycoside fragment from Quil A.

8. Use according to claims 7, wherein the iscom particle comprises subfragment A and /or subfragment C of Quil A.

9. Composition comprising at least one iscom particle and one or more living micro-organisms.

10. Composition according to claim 9, wherein the living micro-organism is a virus.

11. Composition according to any of claims 9-10, further comprising one or more killed or inactivated micro-organisms.

12. Composition according to any of claims 9 -11, further comprising one or more antigenic molecules.

13. Composition according to any of claims 9 -12, wherein the iscom particle comprises at least one glycoside fragment from Quil A.

14. Composition according to any of claims 9 -13, wherein the iscom particle comprises subfragment A and /or subfragment C of Quil A.

15. Composition according to any of claims 9-14, further comprising a pharmaceutically acceptable carrier, diluent, excipient or additive.

16. Kit of parts comprising at least one compartment containing at least one living organism and at least one compartment containing at least one iscom particle.

17. Kit of parts according to claim 16, further comprising at least one inactivated micro-organism, which may be present in a further compartment or in the same compartment as the at least on compartment containing the at least one iscom particle.

Abstract.

The present invention relates to the use of iscom particles as adjuvant for preparing of an antigenic composition, which comprises live micro-organisms and a composition comprising at least one iscom particle and one or more living micro-organisms. It also relates to a kit of parts comprising at least one compartment containing at least one living organism and at least one compartment containing at least one iscom particle.

This Page is inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ BLACK BORDERS
- ☒ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLORED OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REPERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning documents *will not* correct images problems checked, please do not report the problems to the IFW Image Problem Mailbox